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EXPERIMENTAL STUDY

Effect of Pingan Fang, a Traditional Chinese Medicine compound, on behavioral sensitization and conditioned place preference induced by ethanol in mice

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Abstract

OBJECTIVE: To observe the effect of Pingan Fang (PG) on behavioral sensitization and conditioned place preference (CPP) induced by ethanol in mice, and to determine the intervention mechanism of PG on alcohol addiction.

METHODS: A behavioral sensitization mouse model induced by ethanol was established to observe the effect of PG on the development and expression of behavioral sensitization induced by ethanol by recording the spontaneous activity of mice. The resident time of mice in a white box was measured to evaluate the effect of PG on developing CPP induced by ethanol. Concentrations of dopamine (DA), Glutamate (Glu), and ã-aminobutyric acid (GABA) in the corresponding mesolimbic region of mice were determined by enzyme-linked immunosorbent assay.

RESULTS: Although PG did not alter spontaneous activity in mice, it reduced the growth of spontaneous activity stimulated by ethanol. The residence time in the white box after-ethanol-training of mice in CPP experiments was decreased.

CONCLUSION: Our data suggested that PG blocked the development and expression of behavioral sensitization induced by ethanol and the development of CPP in mice. The mechanism might be related to the decreased content of DA and Glu and increased content of GABA in the mesolimbic dopamine system. This suggests that PG might be useful for the prevention and treatment of alcohol addiction.

Key words: Ethanol; Pingan Fang; Behavioral Sensitization; Conditioned place preference; Dopamine; Glutamic acid; Aminobutyrates

INTRODUCTION

Alcohol dependence (AD) as defined by the American Psychiatric Association in the Diagnostic and Statistical Manual of Mental Disorders,[1] is characterized by increased alcohol tolerance, impaired ability of control over drinking, and drinking regardless of consequences. Alcohol dependence is a grievous public health problem that often results in social,medical and eco-

nomic consequences throughout the world.[2] Alcoholism affects nearly 10 percent of the population and results in social problems,considerable morbidity and mortality, and high health care costs.[3,4]

People have an increasing interest in drug therapy for alcohol dependence,[5,6] and the most important rationale for this therapy is based on the growing understanding of the neurobiology of AD. Advances in neurobiology have identified which neurotransmitter systems initiate and maintain the drinking of alcohol, including dopamine (DA), gamma-amino butyric acid (GABA), glutamic acid (Glu), cholinergic systems, 5-hydroxytryptamine systems and endogenous opioid peptide system.[7,8] Pharmacologic modification of these neurotransmitters or their receptors may modify dependence and these systems are potential targets for drug therapy in the treatment of AD. However, currently in the USA, there are four medications approved by the Food and Drug Administration (FDA) to treat AD: disulfiram, naltrexone, and acamprosate. Previous data suggest that their overall efficacy is modest.[9] Therefore, there is a critical need to identify new medications that may be effective in treating AD individuals.

AD is a neurodegenerative disease and studies have shown that brain damage is a common and potentially severe consequence of long-term and heavy alcohol consumption; even mild-to-moderate drinking can adversely affect cognitive functioning.[10] Persistent cognitive impairment can contribute to poor job performance in adult alcoholics, and can interfere with learning and academic achievement in adolescents with an established pattern of chronic heavy drinking.[11] Chinese herbal medicines are the most commonly used Traditional Chinese Medicines. Tianma (*Rhizoma Gastrodiae*) is a well-known herb that has been used to treat neurological disorders in East Asian countries for centuries.[12] Tianma (*Rhizoma Gastrodiae*) has been shown to increase extracellular GABA levels in Sprague-Dawley rats, thus enhancing GABA ergic neurotransmission and decreasing glutamate levels.[13] Gouteng (*Ramulus Uncariae Rhynchophyllae cum Uncis*) prescribed as a Traditional Chinese Medicine is used to treat ailments of the central nervous system. Research shows that Rhynchophylline, an active components of Gouteng (*Ramulus Uncariae Rhynchophyllae cum Uncis*), reduced the spontaneous activity and DA concentration in the cortex, amygdala, and spinal cord and protected neurons from damage induced by DA.[14,15] Baishao (*Radix Paeoniae Alba*),[16] which has been used for over 1500 years in China because of its effects on nourishing yin, replenishing blood of the liver, nourishing liver-yin to calm the liver and suppressing hyperactivity of yang. Therefore, we used Tianma (*Rhizoma Gastrodiae*), Gouteng (*Ramulus Uncariae Rhynchophyllae cum Uncis*) and Baishao (*Radix Paeoniae Alba*) to form a new compound called "Pingan Fang" (PG), which has been used for ten years to treat AD in the Affiliated Hospital of Chengdu University of Traditional Chinese Medicine.

The behavioral sensitization model and the Conditioned Place Preference (CPP) model are two widely used models used for drug addiction research. The behavioral sensitization animal model was found to be closely related to drug addiction and drug craving in humans[17,18] and the CPP model is an indicator of the rewarding effects of drugs. Research showed that ethanol induces a long duration of behavioral sensitization in mice,[19,20] and alcoholics and offspring of alcoholics exhibit reduced behavioral sensitization by ethanol.[21] The above evidence indicates that behavioral sensitization is an important experimental model for the study of alcohol addiction. CPP is a classic model to observe material reward and spiritual dependence, as shown by changes of natural preference after drug training. In this study, we used behavioral sensitization and CPP induced by ethanol in animals to observe the influence of PG on ethanol-induced behavioral sensitization and CPP, to evaluate the effects of PG in the prevention and treatment of alcohol addiction and provide a theoretical basis of PG for treating AD.

MATERIALS AND METHODS

Animals

Three-month-old male Kunming mice weighing (22 ± 3) g were provided by Chengdu Dashuo Animal Experimental Company (Chengdu, China) and were maintained in a specific pathogen free environment. Mice were housed under standard conditions of a 12 h light/dark cycle (lights on from 7: 00 to 19: 00), 22-26 ℃ and 40%-70% humidity. Food and drink were provided ad libitum. All efforts were made to minimize animal suffering and to keep the number of animals used to a minimum. This study was approved by the Chengdu University of Traditional Chinese Medicine Ethics Review Committee and conducted in accordance with the internationally accepted principles for laboratory animal use and care according to the US guidelines (NIH National Institutes of Health Publication No. 85-23, revised in 1985).

Drugs

Saline and ethanol (concentration 96%) solutions were prepared with saline (15% , v/v in 0.9% NaCl) and stored at 4 ℃ . Intragastric (i.g.) administration of PG = 18 g/kg (M g/60 kg × 9 g/kg, M refers to dose of Chinese medicine and 60 kg is the human adult standard weight). Ethanol i.g. administration dose was 2.2 g/kg. Preliminary experiments were used to observe the effect of doses of 1.8, 2.0, 2.2 and 2.4 g/kg. We found 2.2 g/kg of ethanol had a minimum effect on the spontaneous activity of mice.

Reagents and instruments

The following instruments and reagents were obtained: ZZ-6 independent activity tester (Chengdu Thai Union Technology Co., Ltd., Chengdu, China); CPP

experiment instrument (model ZH-CPP, Anhui Zenghua Biological Instrument Equipment Co., Ltd., Anhui, China); Computer (model TFT185W80PS; Guanjie Display Technology Co., Ltd., Xiamen, China); Microplate reader (model Multlskan Mk3; Thermo Fisher Scientific Instrument Co., Ltd., Shanghai, China); Youpu ultra-pure water production system (model UPH-Ⅱ-10T, Chengdu ultra-pure Science and Technology Co., Ltd., Chengdu, China); Electronic constant temperature water bath (model DZKW-4,Beijing Zhongxingweiye Instrument Co., Ltd., Beijing, China); and Rat DA ELISA kit (Kit Item: E-30236); Rat Glu ELISA kit (Kit Item: E-E-31033) and Rat GABA ELISA kit (Kit Item: E-30324) (produced by Abcam, imported and packaged by Beijing Yonghui Biotechnology Co., Ltd., Beijing, China).

Preparation of PG

The formula of PG (one dose) is shown in Table 1. These following herbs were purchased from Sichuan Chinese Herbs Co., Ltd., (Sichuan, China): Tianma (*Rhizoma Gastrodiae*, lot#2013010426), Gouteng (*Ramulus Uncariae Rhynchophyllae cum Uncis*, lot# 2013012121), Baishao (*Radix Paeoniae Alba*, lot# 2013120726). All of these were accredited by a pharmacologist, Prof. Yan Zhuyun, according to the Pharmacopoeia of the People's Republic of China (2010). [22] Their voucher specimens are deposited at the Affiliated Hospital of Chengdu University of Traditional Chinese Medicine (Chengdu, China).

The extraction process of PG followed a traditional method as follows: Tianma (*Rhizoma Gastrodiae*) 30 g, Gouteng (*Ramulus Uncariae Rhynchophyllae cum Uncis*) 60 g, and Baishao (*Radix Paeoniae Alba*) 30 g were added to 1.5 volumes of water, soaked for 30 min and decocted 3 times, 20 min for the first time and 30 min for the second time and third times. The decoctions were combined, filtered and concentrated to 1.5 g/mL (1 mL extract contained 1.5 g of herbal mixture).

Behavioral sensitization test methods and procedures

The behavioral sensitization animal models induced by ethanol in mice were performed as previously described, [22,23,24] with some alterations as shown in Table 1.

Habituation phase (days 1-3)

Before medication, mice were weighed 1 h before they were placed in a test chamber. Animals were tested in the test chamber for 15 min immediately after saline administration. This procedure was repeated every day, during a 3-day period. The purpose was to let the mice adapt to the test device to exclude the influence of the environment and gastric administration on their spontaneous activity and to record their baseline spontaneous activity.

Treatment phase (days 6-15)

After 48 h of baseline measurement, 120 mice were randomly divided into four groups using the random number table method 30 min before administered PG (Z: 18 g/kg) or saline (S) (i.g.), followed by gavage with saline or ethanol (2.2 g/kg). The four groups were: saline + saline (S + S, n = 30), saline + ethanol (S + E, n = 30), saline + PG (Z + S, n = 30), and PG + ethanol (Z + E, n = 30). The animals were placed in

Table 1 Experimental groups and treatments. Pre-treatment drug administration (i.g.) was given 30 min before treatment

Group	Treatment phase		Challenge phase		
	Days 6, 8, 10, 12 and 14		Day 18 (saline challenge)	Day 21 (drug challenge)	
	Pre-treatment	Treatment	Treatment	Pre-treatment	Treatment
S+S	Saline	Saline	Saline	Saline	Ethanol
				Z	Saline
				Z	Ethonal
Z+S	Z	Saline	Saline	Saline	Ethanol
				Z	Saline
				Z	Ethonal
S+E	Saline	Ethanol	Saline	Saline	Ethanol
				Z	Saline
				Z	Ethonal
Z+E	Z	Ethanol	Saline	Saline	Ethanol
				Z	Saline
				Z	Ethonal

Notes: S + S: (saline + saline, n = 30) treated with saline and saline; Z + S: (PG + saline, n = 30) treated with PG (18 g/kg) and saline; S + E: (saline + ethanol, n = 30) treated with saline and ethanol (2.2 g/kg); and Z + E: (PG + ethanol 2.2 g/kg, n = 30) treated with PG (18 g/kg) and ethanol (2.2 g/kg). S: saline; E: ethanol; Z: PG (Pingan Fang).

the test instrument for 15 min, immediately after ethanol (or saline) administration. This procedure was repeated every other day, during a 10-day period (five tests). Forty-eight hours after the end of the treatment, the challenge phase started.

Challenge phase (day 18)

After 48 h being drug-free, mice in the group were randomly divided into three subgroups based on the last time results of spontaneous activity from each group. The challenge phase included saline challenge and drug challenge. In the saline challenge, mice were placed in the test chamber for 15 min, immediately after saline administration. After 48 h, the ethanol challenge began. Immediately after three subgroups received ethanol, PG or PG + ethanol, mice were placed in the spontaneous activity instrument for 15 min.

Specimen collection and detection

Mice in four subgroups (S + S + E, n = 10; S + E + E, n = 10; S + E + Z + E, n = 10; Z + E + E, n = 10) were decapitated immediately after testing. Brain tissues were placed on an ice pillow after dissection, and the mesolimbic areas of the brain were removed and washed with ice-cold distilled water. Then they were placed in a 5 mL glass homogenizer pre-filled with ice-cold saline, homogenized for 3 min, then centrifuged at 50,005 × 10^3 rpm at freezing temperature for 10 min. Supernatants were collected. ELISAs were performed according to the manufacturer's guidelines. Final values were measured as the absorbance at 450 nm wavelength (OD).

CPP experiment

In this study, the experimental procedure was divided into a pre-adaptation phase, training phase and expressing testing. Light, color, odor, and other environmental conditions in the box were consistent throughout the experiment. The CPP animal model was induced by ethanol as previously described,[25] with some adaptions as shown in Table 3.

Pre-adaptation phase (day-2-day 0)

Mice were placed in the middle of the CPP box and allowed free movement for 15 min. This procedure was repeated every day, during a 3-day period and saline was administrated every day to eliminate the effect of the experimental operation on mice. The residence time of mice in three boxes on the 3rd day was recorded. The residence time of the mice in different regions within 15 min was recorded as an index of natural preference. The long-time side of the box was the non-medicine box and the other side was the medicine box. Under the condition of this experiment, mice had a natural preference for black. Therefore, we adopted an experimental design with bias and used a white chamber as the medicine chamber and black chamber as the non-medicine chamber.

Training phase (day 1-10)

After the pre-adaptation phase, mice were randomized into four groups by the random number table method, with 12 per group: (saline: S + S, ethanol: S + E, PG: S + Z, and PG + ethanol: Z + E). On odd-numbered days animals were administered saline or PG i.g. (18 g/kg), then administered saline or ethanol i.g. (2.2 g/kg) after 30 min, and placed in the medicine box for 1 h. On even-numbered days, animals were administered saline i.g. before being placed in the non-medicine box for training. There were five medicine/saline training cycles in total. The training time was fixed between 8 points to 9 points every morning.

Testing phase (day 11)

Thirty min after saline was administered, mice were placed in the middle box for 15 min. The residence time of mice in medicine box was recorded.

Statistical analysis

All variables were expressed as the mean ± standard deviation ($\bar{x} \pm s$). Statistical analysis was carried out using SPSS 17.0 software (Chicago, IL, USA). Sample mean differences between the two groups were compared by independent t-test. Comparison of CPP in mice in the medicine box before and after was determined by independent t-test. Means of multiple groups were compared by One-Way Analysis of variance (ANOVA). Least significant difference (LSD) or Bonferroni method was used for pairwise comparisons; with time variable data, the single factor analysis of variance for repeated measurements was used and Bonferroni post-tests method was used for multiple comparisons. The times of challenge phase activity were compared using analysis of covariance (ANCOVA) and LSD method for multiple comparisons. All P values were two-sided tests. $P < 0.05$ was considered statistically significant.

RESULTS

Baseline of locomotor activity

A one-way ANOVA showed that the baseline of locomotor activity in the habituation test was similar between all groups (Figure 1).

Effect of repeated dose on locomotor activity of mice

The independent t-test analysis showed that mice in the S + E group had higher levels than in the S + S group in test 1 (t = 3.483, P = 0.001, n = 30), test 2 (t = 4.305, P = 0.000, n = 30), test 3 (t = 12.781, P = 0.000, n = 30), test 4 (t = 19.549, P = 0.000, n = 30) and test 5 (t = 17.635, P = 0.000, n = 30). Mice in the S + E group had higher levels than the Z + S group in test 2 (t = 4.955, P = 0.000, n = 30), test 3 (t = 12.119, P = 0.000, n = 30), test 4 (t = 19.538, P = 0.000, n = 30), and test 5 (t = 19.368, P = 0.000, n = 30). A one-way ANOVA showed that in the tests 4 and

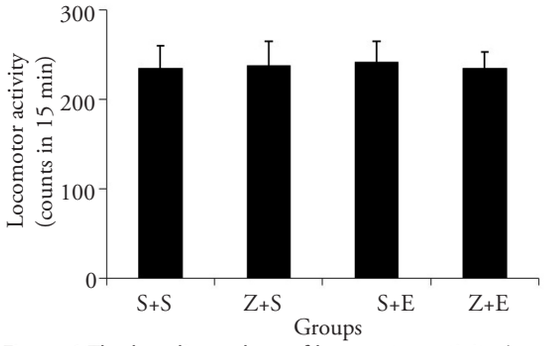

Figure 1 The baseline values of locomotor activity (counts in 15 min) in the habituation test

Mice were pre-treated with PG (i.g.) (18 g/kg) or saline (i.g.) 30 mins before the test. S + S: (saline + saline, *n* = 30) treated with saline and saline; Z + S: (PG + saline, *n* = 30) treated with PG (18 g/kg) and saline; S + E: (saline + ethanol, n = 30) treated with saline and ethanol (2.2 g/kg); and Z + E: (PG + ethanol 2.2 g/kg, *n* = 30) treated with PG (18 g/kg) and ethanol (2.2 g/kg). S: saline; E: ethanol; Z: PG (Pingan Fang).

5, mice in the S + E (F = 90.692, *P* =0.000, *n* = 30) and Z + E (F = 5.772, *P* = 0.000, *n* = 30) groups had higher activity levels than in tests 1, 2 and 3 (Figure 2). In the 4th and 5th tests, mice in the Z + E group had higher levels than the S + S group (*t* = 2.769, *P* = 0.008; *t* = 3.018, *P* = 0.004, *n* = 30) and the Z + S group (*t* = 3.889, *P* = 0.000; *t* = 3.624, *P* = 0.001, *n* = 30) (Figure 2).

Effect of PG on behavioral sensitization induced by ethanol in mice

A one-way ANOVA showed that the ethanol challenged group had higher locom-otor activity levels than the Pingan Fang challenged group (F = 10.100, *P* = 0.000). The one-way ANOVA detected higher locomotor activity levels in the S + E treated group compared with all other groups during saline challenge (F = 6.048, *P* = 0.002) (Figure 3).

Effect of PG on DA, Glu, and GABA levels in the brain tissues of mice

One-way ANOVA demonstrated that the concentration of DA and Glu in the S + E + E group was higher than in the other three groups (F = 52.276, *P* = 0.000; F = 13.594, *P* = 0.000, *n* = 10). There was no difference in the concentrations of DA and Glu between the S + S + E group and the PG group. The concentration of GABA in the S + E + E group was higher than in the other three groups (F = 5.597, *P* = 0.000, *n* = 10) (Table 2).

Natural preference effect experiments in mice

The independent *t*-tests showed that the residence time of mice in the black chamber was 520.88 ± 46.716 s in pre-experiments and the time in the white chamber was 223.04 ± 38.511 s. The residence time of mice in the black chamber was higher than in the white chamber (*t* = 34.082, *P* = 0.000, *n* = 48). This suggested that mice had a natural preference for the black sides of the metal bottom grid box. Therefore, we adopted an experimental design with bias and used the white chamber as the medicine chamber and the black chamber as the non-medicine chamber .

Effect of PG on ethanol-induced CPP development phase in mice

After a 10-day period (five tests), one-way ANOVA showed the time spent in the medicine chamber was higher in the S + E group compared with the other three groups (F = 201.431, *P* = 0.000) and higher than during the preconditioning phase (*t* = 14.314, *P* = 0.000) (Figure 4).

DISCUSSION

Behavioral sensitization performance is characterized by an increase in locomotor activity after the continu-

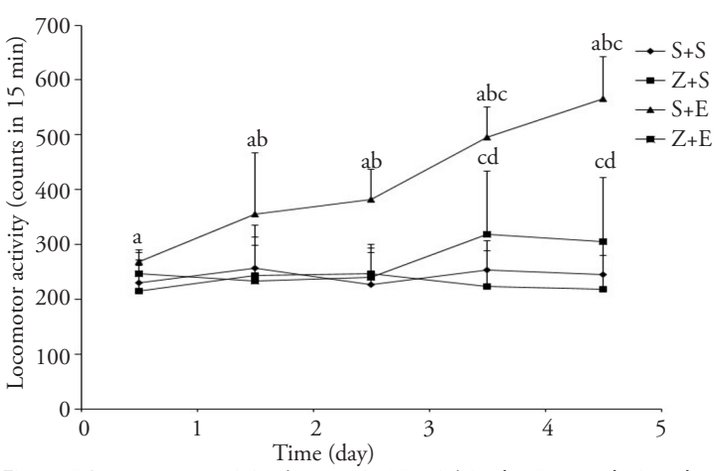

Figure 2 Locomotor activity (counts in 15 min) in the 5 tests during the 10-day period of treatment, immediately after ethanol or saline administration (i.g.)

Mice were pre-treated with PG (i.g.) (18 g/kg) or saline (i.g.) 30 min before the test. S + S: (saline + saline, *n* = 30) treated with saline and saline; Z + S: (PG + saline, *n* = 30) treated with PG (18 g/kg) and saline; S + E: (saline + ethanol, *n* = 30) treated with saline and ethanol (2.2 g/kg); and Z + E: (PG + ethanol 2.2 g/kg, *n* = 30) treated with PG (18 g/kg) and ethanol (2.2 g/kg). S: saline; E: ethanol; Z: PG (Pingan Fang). $^{a}P < 0.05$, compared with S + S group in tests 1, 2, 3, 4 and 5. $^{b}P < 0.05$, compared with the Z + S group in tests 2, 3, 4, and 5.$^{c}P < 0.05$, compared with tests 1, 2, and 3. $^{d}P < 0.05$, compared with the S + S group and Z + S group in tests 4, and 5.

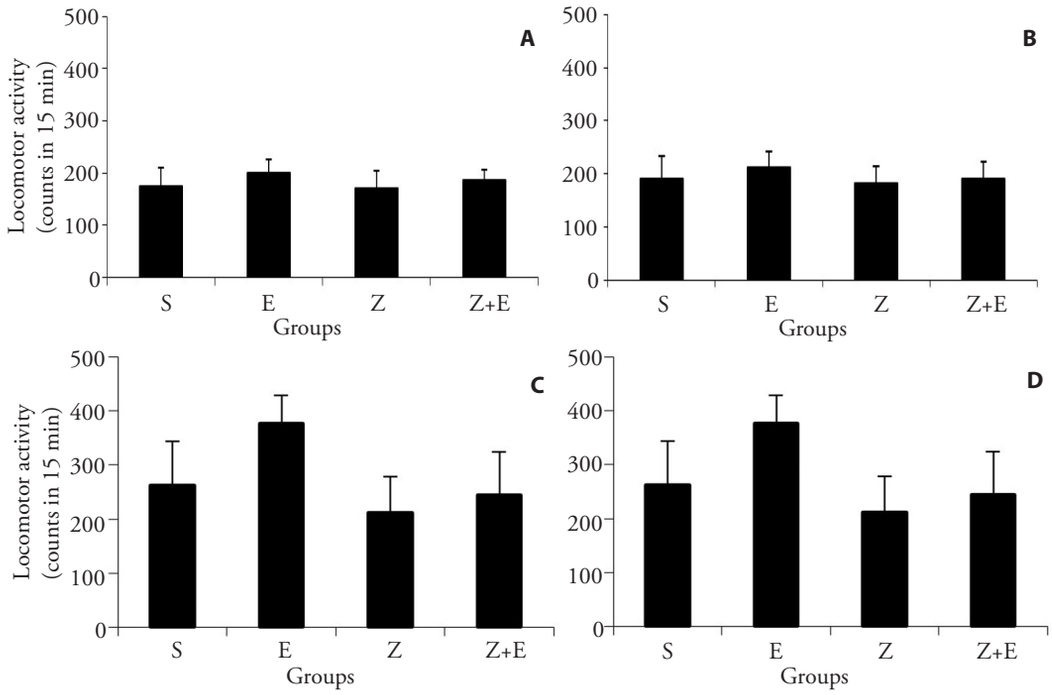

Figure 3 Locomotor activity (counts in 15 min) in challenge tests

A: group previously treated (i.g.) with S + S (saline and saline); B: group previously treated (i.g.) with Z + S (PG: 18 g/kg and saline); C: group previously treated (i.g.) with S + E (saline and ethanol: 2.2 g/kg); D: group previously treated (i.g.) with Z + E (PG: 18 g/kg and ethanol: 2.2 g/kg). S: saline; E: ethanol; Z: PG (Pingan Fang). Different drugs were used (i.g.) in each "drug challenge". S: challenged with saline and saline (n = 30); E: challenged with saline and ethanol (2.2 g/kg) (n = 10); Z: challenged with PG (18 g/kg) and saline (n = 10); Z + E: challenged with PG (18 g/kg) and ethanol (2.2 g/kg) (n = 10). The mice were tested in the locomotor activity cages for 15 min immediately after ethanol (2.2 g/kg) or saline administration. [a]P < 0.05, compared with those from all other treatment groups with saline challenge; [b]P < 0.05, compared with all the other groups with drug challenges (P < 0.05).

Table 2 Mean DA, Glu, and GABA levels in the brain tissue of mice ($\bar{x} \pm s$)

Group	DA (ng/L)	Glu (nmol/L)	GABA (ng/mL)
S+S+E	58.3±3.02	124.4±1.1	14.6±1.4
S+E+E	72.5±4.52[a]	134.2±3.7[b]	12.7±1.3[c]
S+E+Z+E	60.8±1.5	126.6±7.3	13.7±0.4
Z+E+E	56.1±3.0	123.7±1.0	13.7±1.2

Notes: S + S + E: treated (i.g.) with saline and saline in the treatment phase, then treated (i.g.) with ethanol (2.2 g/kg) in the challenge phase (n = 10); S + E + E: treated (i.g.) with saline and ethanol (2.2 g/kg) in the treatment phase, then treated (i.g.) with saline and ethanol (2.2 g/kg) in the challenge phase (n = 10); S + E + Z + E: treated (i.g.) with saline and ethanol (2.2 g/kg) in the treatment phase, then treated (i.g.) with PG (18 g/kg) and ethanol (2.2 g/kg) in the challenge phase (n = 10); Z + E + E: treated (i.g.) with PG (18 g/kg) and ethanol (2.2 g/kg) in the treatment phase, then treated (i.g.) with saline and ethanol (2.2 g/kg) in the challenge phase (n = 10). DA: dopamine; Glu: Glutamate; GABA: ã-aminobutyric acid; S: saline; E: ethanol; Z: PG (Pingan Fang). [a]P < 0.01, compared with the other three groups; [b]P < 0.05, compared with the other three groups, [c]P < 0.05, compared with the other three groups.

ous use of addictive drugs.[26] It can be seen after using cocaine, morphine and nicotine, and is the key to drug addiction.[18,27] According to the drug addiction motivation sensitization theory, behavioral sensitization plays an important role in forced drug use, drug-seeking behavior and behavior of relapse after withdrawal.[28] The main mechanism of behavioral sensitization is the adaptive change of the central nervous system and synaptic plasticity,[18] which is a recognized model in the study of drug addiction. Repeated activation of the mesolimbic dopamine system (MDLS) and increased release of DA to induce the rewarding effect of ethanol is considered to be key to the formation of behavioral sensitization. A study found that ethanol increased the concentrations of DA in the accumbens nucleus (NAc),[29] and the release of DA decreased after the withdrawal of ethanol.[30] Experiments have shown that in the formation of behavioral sensitization, there is an increased output of Glu from the prefrontal cortex, amygdala, hippocampus, and other limbic brain regions to the ventral tegmental area and NAc.[31] Evidence showed that the excitatory pathway mediated by Glu played an important role in the pathogenesis of alcohol dependence, and the use of drugs against Glu such as acamprosate had an effect in the treatment of alcohol addiction.[32] GABA is the major inhibitory neurotransmitter in central nervous system. It inhibits the release of mesolimbic DA to weaken the effect of cocaine, heroin, nicotine, alcohol and other addictive drugs.[33] Shuchang *et al* [13] showed that GE increased the extracellular GABA lev-

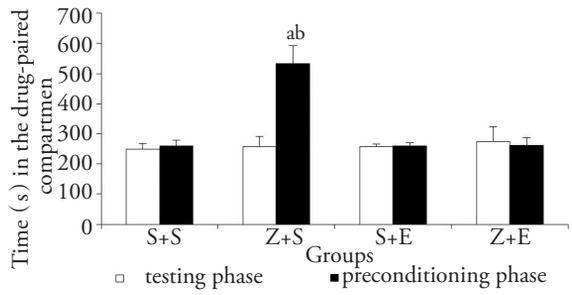

Figure 4 Effect of PG on the development of ethanol-induced CPP in mice

S + S: treated (i.g.) with saline and saline in the training phase; S + E: treated (i.g.) with saline and ethanol (2.2 g/kg) in the training phase; S + Z: treated (i.g.) with saline and PG (18 g/kg) in the training phase; Z + E: treated (i.g.) with PG (18 g/kg) and ethanol (2.2 g/kg) in the training phase, then the four groups were all treated (i.g.) with saline in the testing phase. S: saline; E: ethanol; Z: PG (Pingan Fang). $^{a}P < 0.05$, compared with the other three groups in the testing phase. $^{b}P < 0.05$, compared with the preconditioning phase.

els in Sprague-Dawley rats, and consequently enhanced GABAergic neurotransmission and decreased Glu levels. In addition, it has been reported that GE reduced the content of DA in the brain.[34] A study also showed that rhynchophylline reduced the DA concentrations in the cortex, amygdala, and spinal cord, enhanced sedatives and reduced spontaneous activity; however, its hypnotic effect on mice was not observed.[14] Zhixian and other researchers[35,36] found that rhynchophylline adjusted the abnormal changes of amino acid neurotransmitter content in the brains of rats induced by amphetamine, thereby eliminating place preference and behavioral sensitization.

In the CPP experiment we found that the ethanol groups of trained mice stayed in the white box longer than the mice without training, and for significantly longer times than those in the saline groups, which reflected the psychological dependence of alcohol. The residence time of mice from the saline + PG group in the white box was not significantly different before or after training, suggesting PG itself does not induce the formation of CPP in mice and does not have a psychological dependence. The residence time of mice from the PG + ethanol group in the white box was not significantly different before or after training, which indicated that PG inhibited the formation of CPP induced by ethanol. However, it is not clear whether it has an inhibitory effect on the expression of CPP induced by ethanol.

In summary, we showed that PG inhibited behavioral sensitization reduced by ethanol in mice and the acquisition and expression of CPP. The inhibition effect may have a relationship with the function of PG that affects the mesolimbic system, down-regulating DA and Glu content and increasing GABA content. Thus we infer that PG could prevent forced medication behavior, drug-seeking behavior after withdrawal, the relapse behavior of alcohol addicts, and have intervention effects for alcohol dependence. This study provided an experimental basis for the mechanism of PG for the intervention of alcohol addiction. However, future studies are needed to explore the exact mechanisms of PG on anti-alcohol addiction.

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